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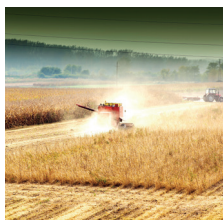
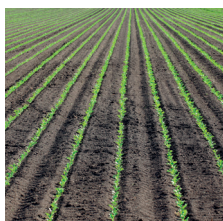
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European Coexistence Bureau (ECoB)

Best Practice Document for the coexistence of genetically modified soybean crops with conventional and organic farming

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Abstract

The present technical report deals with coexistence issues of genetically modified (GM) soybean cultivation with non-GM soybean and honey production in the EU. The Technical Working Group (TWG) for Soybean of the European Coexistence Bureau (ECoB) analysed the possible sources for potential GM cross-pollination and admixture and agreed on the best practices for coexistence. The terms of reference for this review are presented in Section 1. The scope of the Best Practice Document is coexistence in soybean crop production in the EU. It includes the coexistence between GM soybean cultivation and honey production but excludes coexistence in seed production.

The ECeB TWG for Soybean conducted two meetings, one in May 2013 and one in February 2014 examining the state-of-art knowledge from scientific literature, research projects and reports, as well as empirical evidence provided by already existing segregation systems in soybean production. The information reviewed amounts to a total of 123 references listed in this report. The report summarises the review of available information on adventitious GM presence in soybean crop production covering seed impurities, cultivation, outcrossing to non-GM soybeans, and volunteers. The process management during sowing, harvesting, transportation, drying and storage on farm is also reviewed. Additionally the report analyses existing studies dealing with the presence of soybean pollen in honey. Finally, the TWG for Soybean reviewed the state of the art for the detection and identification of traces of GM soybean material in non-GM soybean harvests and honey.

Based on this review, the members of the TWG Soybean submitted proposals for best management practices, which form the basis of the agreed consensus recommendations presented in Section 8.



European Coexistence Bureau (ECoB)

Best Practice Document for the coexistence of genetically modified soybean crops with conventional and organic farming

Ivelin Rizov and Emilio Rodrigues-Cerezo.

2015

Joint Research Centre

This best practice document is the result of work carried out by the European Coexistence Bureau – Technical Working Group for Soybean, consisting of the following European Commission staff and experts nominated by EU Member States:

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Executive summary

The present technical report deals with coexistence issues of genetically modified (GM) soybean cultivation with non-GM soybean and honey production in the EU. The Technical Working Group (TWG) for Soybean of the European Coexistence Bureau (ECoB) analysed the possible sources for potential GM cross-pollination and admixture and agreed on the best practices for coexistence. The terms of reference for this review are presented in Section 1. The scope of the Best Practice Document is coexistence in soybean crop production in the EU. It includes the coexistence between GM soybean cultivation and honey production but excludes coexistence in seed production.

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1. Introduction

1.1. Legal background

Agricultural practices are conducted in an open environment and enable the possibility of interactions among different production systems. The European coexistence legislative framework was created to ensure that cultivation of genetically modified (GM) crops is carried out in a way that allows different agricultural systems to co-exist side by side in a sustainable manner. The legislative basis in the EU for the coexistence of GM and non-GM crops is established by the relevant legislation for the release of genetically modified organisms (GMOs) into the environment (Directive 2001/18/EC³) and the food and feed legislation for the labelling requirements in case of GM admixtures (Regulation No 1829/2003⁴). Both parts of legislation provide a harmonised approach to ensure strict control of placing GMOs on the EU market. All GMOs and food and feedstuffs derived from them have to be clearly labelled to ensure freedom of choice for the customers. As an exception to this labelling requirement, the European legislation takes into consideration the low level presence of technically unavoidable or adventitious traces of GM material.

The Regulation (EC) No 1829/2003 establishes a threshold of 0.9% for food and feed. This labelling threshold should also be valid for admixture of GM pollen in honey, following the European Commission proposal from 21st of September 2012 for amendment of Council Directive 2001/110/EC⁵ to clarify the status of pollen in honey as a natural constituent of honey and not as an ingredient, which is in line with international FAO and WHO standards. The European parliament endorsed draft rules defining pollen as a natural constituent of honey similar to the Commission proposal during its Plenary Session on 15th of January 2014.

Directive 2008/27/EC⁶, which amended Directive 2001/18/EC, established the same threshold of 0.9% for commodities intended for direct processing, below which traces of market approved GM products do not require labelling. For seed lots such an exclusion from the labeling rules is not foreseen and all marketed seeds in the EU with GM impurities must be labeled, regardless of its level.

These labelling rules are also valid for organic products, including food and feed, according to Regulation (EC) No 834/2007⁷.

Commission Recommendation 2010/C 200/01⁸ provides guidelines for the development of national coexistence measures to avoid the unintended presence of GMOs in conventional and organic crops and replaces Commission Recommendation 556/2003⁹. The recommendation considers that the market demand for particular food crops may result in economic damage to operators who would wish to market them as not containing GMOs, even if GMO traces are present at a level below 0.9%. Therefore, Member States (MS) may establish different thresholds for the adventitious and technically unavoidable presence of GMO in non-GM harvests, taking into account the demands of the consumers and their market. The Recommendation also takes into consideration the extreme diversity of European farming systems, natural and economic conditions and the experience gained in coexistence over recent years, and clarifies that, under certain climatic and/or agronomic conditions, MS may exclude GMO cultivation from large areas, if other measures are not sufficient to ensure coexistence at a regional level.

3 Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39

4 Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23

5 Council Directive 2001/110/EC of 20 December 2001 relating to honey. OJ L 10, 12.1.2001, p. 47.

6 Directive 2008/27/EC of the European Parliament and of the Council of 11 March 2008 amending Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, as regards the implementing powers conferred on the Commission, OJ L 81, 20.3.2008, p. 45–47

7 Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. OJ L 189, 20.7.2007, p. 1–23.

8 Commission Recommendation of 13 July 2010 on guidelines for the development of national co-existence measures to avoid the unintended presence of GMOs in conventional and organic crops (2010/C 200/01), OJ C 200, 22.7.2010, p. 1–5

9 Commission Recommendation 556/2003 of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming. OJ L 189, 29.7.2003, p. 36.

1.2. The role of the European Coexistence Bureau

The subsidiarity approach adopted in the EU for the implementation of coexistence regulations, in reflection of the heterogeneity in agricultural practices and legal environments among the MS, has led to the definition of various coexistence measures among MS.

However, the European Commission (EC) retains important roles in developing national coexistence regulations. One of them is the provision of specific technical advice to the MS on how to develop coexistence measures, and this is done through the European Coexistence Bureau (ECoB) hosted and managed by the European Commission's Joint Research Centre (JRC).

The activities of the ECoB are supported by crop-specific Technical Working Groups (TWG) consisting of experts nominated by the EU MS. Their main task is to develop Best Practice Documents (BPDs).

The first TWG for maize crop production started its work in 2008. The TWG for maize has developed three BPDs for:

- Coexistence of GM maize crop production with conventional and organic farming (Czarnak-Kłós and Rodríguez-Cerezo, 2010);
- Monitoring efficiency of coexistence measures in maize crop production (Rizov and Rodríguez-Cerezo, 2014); and
- Coexistence of GM maize and honey production (Rizov and Rodríguez-Cerezo, 2013).

The second TWG, for soybean, was established in 2013.

1.3. Scope of the Best Practice Document

The TWG for soybean of the ECoB was asked to propose, based on current scientific knowledge and agricultural practices, a set of best agricultural management practices that will ensure coexistence of GM soybean with conventional and organic soybean while maintaining economic and agronomic efficiency of the farms. The TWG was also asked to examine the issue of coexistence between GM soybean cultivation and honey production in the EU. The scope of the BPD is coexistence in the cultivation of soybean in the EU and not elsewhere.

The document considers both the need for compliance with the regulated labelling threshold of 0.9% as well as with lower thresholds of adventitious presence of GM material which may be required by operators in some markets. This scope is in line with the Commission Recommendation of 13 July 2010 on guidelines for the development of national coexistence measures.

The document exclusively considers GM soybeans with a single gene transformation event.

For the purpose of this document it is assumed that the coexistence measures proposed would be implemented by the GM soybean producers. All these measures should be proportional as well as technically and economically consistent.

2. Soybean biology

Soybean [*Glycine max* (L.) Merrill] is a member of the Fabaceae family, subfamily Papilionoideae, tribe Phaseoleae, genus *Glycine*. The genus *Glycine* is composed of two subgenera, *Glycine* (seven wild perennial species) and *Soja* (annuals) which includes the cultivated soybean (*Glycine max*) and its wild annual counterpart *G. soja* (Newell & Hymowitz, 1983).

The soybean that is currently cultivated originates from China. Soybean is commonly considered as one of the oldest cultivated crops, native to North and Central China (Hymowitz, 1970). The introduction of soybean to Southeast Asia was finalized around the 15th to the 16th century and in Europe before 1713. The introduction to North America took place in 1765 (Cober et al., 2009).

It is assumed that the ancestor of the genus *Glycine* ($x=10$) has undergone tetraploidization approximately 59 and 13 million years ago (Schmutz et al., 2010). However all described species of the genus *Glycine* exhibit normal diploid meiosis and are primarily inbreeders (Cober et al., 2009). Therefore, soybean ($2n=4x=40$) can be considered as an ancient polyploid or paleopolyploid plant (Schmutz et al., 2010). The further evolution of soybean started from a common wild perennial progenitor ($2n=4x=40$) that evolved to a wild annual ($2n=4x=40$) and finally to the domesticated soybean ($2n=4x=40$) (Cober et al., 2009).

2.1. Flower and pollen morphology

Typically for most legumes, flower petals of soybean enclose almost entirely the male and female organs. The soybean flower is papilionaceous, with a tubular calyx of five unequal sepal lobes and a five-parted corolla consisting of posterior standard petal, two lateral wing petals and two anterior keel petals in contact with each other but not fused (Carlson and Larsten, 1987). Soybean inflorescence is a raceme bearing

5–35 flowers, and a single plant may produce up to 800 flowers during its lifetime, but each flower lasts only 1 day (Delaplane and Mayer, 2000). The zygomorphic flowers are white, pink or purple, hermaphrodite and self-fertile. The soybean flowers feature cleistogamy (the flowers of soybean open after pollination), the stigma becomes receptive a day or two before opening of the flower and the pollen is released the night before or the morning of the day the flower opens, resulting in a high rate of self-pollination (Carlson et al., 2004). The stigma is exposed to external influence only after having been nearly exclusively auto-pollinated (Fehr, 1980; Delaplane and Mayer, 2000). The viability of soybean pollen is very limited and does not exceed two to four hours. Fertilization is completed within 10 hours after the opening of the flower.

The pollen development of soybean during various phases of microsporogenesis is sensitive to increased temperature stress (Salem et al., 2007). Djanaguiraman et al. (2013) showed that decreases in pollen in vitro germination by high temperature stress are caused by anatomical changes in pollen, leading to decreased pod set percentage under these conditions.

The soybean pollen grains are spherical in shape¹⁰ with a mean size of 30.4–27.3 μm (Yoshimura, 2011). Kaltchuk-Santos et al. (1993) reported dimorphism in soybean pollen with the normal microspores measured 26.23 μm in diameter and 23.09 μm in distance between two pores, while “P pollens” (pre-mitotic pollen, non-functional gametophyte) had a diameter of 23.87 μm and a distance of 18.49 μm between the pores. In general soybean pollen is among the smallest of all cultivated plants. The size of the soybean plant and the structure of soybean flowers restrict significantly its transportation by wind over long distances (Fehr, 1987; Yoshimura, 2011). The study of Yoshimura (2011) showed little airborne pollen in and around the soybean field and that its dispersal is restricted to a small area. Therefore, wind-mediated pollination appears to be negligible.

¹⁰ www.palдат.org, Austrian melissopalynological website

2.2. Insect impact on cross-pollination

Soybean is exclusively a sexually reproducing, self-pollinating plant usually with a rate of self-pollination higher than 99% (Weber and Hanson, 1961; Caviness, 1966; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007; Anderson and Vicente, 2010) and does not show obligate insect pollination (Rubis, 1970; McGregor, 1976; Ahrent and Caviness, 1994; Wolff, 2000). This high rate of autogamy in soybeans is due to cleistogamy. However, entomophilous (insect) pollination occurs as a consequence of early opening flowers or visits of specialized foraging insect species, in search of pollen and nectar, which are mainly bees (Chiari et al., 2005b). These include species belonging to the genera *Apis*, *Xylocopa* and *Megachile* (*Megachile turugensis* Cockerell), as well as the family *Halictidae* (*Halictus* spp.). For example the conventional soybean cultivar BRS-133 is intensively visited by *Apis mellifera* Africanized honeybees (Chiari et al., 2005a,b). An increase of more than 61% in the number of pods, and more than 58% in yield, in comparison to plants protected against insect visitation, is reported. Furthermore, Robacker et al. (1983) and Milfont et al. (2013) observed yield increases of about 10 to 40% in honeybee-pollinated soybean plants compared to self-pollinated plants, whilst cage inclusion trials have shown up to 15% increase in production (Erickson et al., 1978). However, all the above mentioned studies do not provide clear evidence if the reported effects are caused by cross-pollination or by stimulation of self-pollinating, meaning stimulation of pollen transfer by visiting insects within the flowers. Furthermore the high rate of soybean flower abortion of about 75%, which could potentially be due to poor pollination or to limited resources, suggests that the more important role for honey bees may be in the facilitation of self-pollination, rather than cross-pollination (Delaplane & Mayer 2000), and most cross-pollination occurs between plants in close proximity (a few meters or less; May & Wilcox 1986; Ray et al. 2003). Some soybean cultivars are also visited by thrips and pollinivore predatory species of the order Hemiptera which may also play a role as pollinator.

Abrams et al. (1978), in attempts to investigate the potential impact of honeybees on cross-pollination of cultivated soybean, conducted experiments in which soybeans and colonies of *A.mellifera* were collocated in cages, and reported an outcrossing rate of 7%. Gumisiriza and Rubaihayo (1978) studied the impact of planting density of soybean on insect pollination activities. They reported 4.5% outcrossing in 30 x 30 cm spaced plots while 2.5% and 2.0% were recorded for 40 x 40 cm and 50 x 50 cm spaced plots, respectively. There was no cross-pollination in the 10 x 10 cm plots, suggesting that very close spacing precluded insect pollinators. When plants were grown in adjacent rows 102 cm apart, under favourable environment conditions and the presence of an adequate quantity of pollinators, Ahrent and Caviness (1994) showed an outcrossing rate of 2.5% as maximum for one soybean cultivar of maturity group VI. The range of

cross-pollination for the other investigated cultivars was 0.09% to 1.63%. In a review of male sterile lines, examining the hybridization potential of soybean, Palmer et al. (2001) showed that the presence of pollinators could lead to a maximal cross-pollination of 20%. However, no information about the distance from the pollen source is presented. Thus, although high variation in cross-pollination between cultivars was found, overall outcrossing was low.

According to Roumet and Magnier (1993), insects do not cause random dispersal of pollen since they prefer to move over short distances. Thus, pollination between adjacent plants is common. Moreover, in that particular study insects tended to move within, rather than between, rows.

In addition to existing genotypic variation between varieties, it appears that differences in environmental conditions and the absence of other pollen and nectar sources are responsible for the increased rates of cross-pollination, with climatic conditions (temperature/humidity) and abundance of pollinators playing a determining role (Gumisiriza and Rubaihayo, 1978; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

2.3. Crop biology and cultivation

Soybean is a quantitative short day plant and hence flowers under short day conditions. Anthesis normally occurs in late morning. It represents a summer annual crop with optimum growing temperatures between 20°C and 30°C, thus cultivation is successful in climates with hot summers. Below 20°C and over 40°C the plants grow significantly slower. From sowing to harvesting it takes 80–120 days (OECD, 2000). None of the soybean varieties are frost tolerant, and therefore they do not survive freezing winter conditions.

Soybean is unique among legumes in that its flowering and ripening is controlled not like most plants by the air temperature, but by the day length, the photoperiod. Since day length changes with latitude, most soybean varieties will only produce a good crop in a relatively narrow band of about 250 km from north to south. North of this band, flowering and maturing is delayed; south of this band it is accelerated.

Three types of growth habit can be found amongst soybean cultivars: determinate, semi-determinate and indeterminate (Bernard and Weiss, 1973). Determinate growth is characterised by the cessation of vegetative growth of the terminal bud after generating inflorescences at both axillary and terminal racemes which are blooming at about the same time. Indeterminate genotypes continue vegetative growth upward at the tip of the stem for several weeks after flowering begins lower at the stem. Upper nodes will flower later. Most commercial varieties are indeterminate. Semi-determinate types have indeterminate stems that

terminate vegetative growth abruptly after the flowering period. They typically grow taller and develop well in short growing seasons. Determinate plants complete their growth in height and then produce all the flowers at about the same time. The difference in growth habit is due to two alleles, Dt1 for indeterminate and dt1 for determinate growth, at a single locus (Robinson and Wilcox, 1998). Mature plants of determinate cultivars are about 50% shorter compared to indeterminate cultivars.

Wilcox and Frankenberger (1987) evaluated the growth of five pairs of determinate and indeterminate isolines or related cultivars for three and five planting dates during 3 years. They found a linear decrease in plant height and node number due to delayed planting for indeterminate cultivars but no such response for determinate cultivars. Both plant types responded to delayed planting in the same way in respect of duration of vegetative growth, but not in respect of duration of reproductive growth.

As a result, photoperiodism and temperature response are important in determining areas of cultivar adaptation and temporal segregation of different soybean cultivars. For example determinate soybeans complete their growth cycle before they start to flower and by appropriate selection of different determinate soybean varieties even in one

maturity group it is possible to achieve temporal isolation among different cultivars, if climatic factors determining flowering time (e.g. temperature, daily sunshine duration) are predictable.

The date of transition from vegetative to reproductive development is controlled by day length. The day length's influence on the physiological development of the soybean plant differs between varieties. Relative maturity is a parameter that provides the information of how many days it takes until maturity is reached at given climatic conditions. The classification of soybean varieties in maturity groups is based on the ability of the variety to effectively use the length of the growing season in a region. Soybean varieties are grouped into 13 maturity groups, according to the climate and latitude for which they are adapted. These maturity groups are numbered from 000, 00, 0 and I to X (Kaya et al, 2004).

Because of the diverse European climatic conditions, it is useful to identify geographical zones where agricultural, plant health and environmental (including climatic) conditions are appropriate for soybean cultivation. The geographical zones used are the same as those described in the Regulation on Plant Protection Products¹¹ (Table 1).

Table 1: EU geographic zones (based on the Regulation on Plant Protection Products)

Zone	Geography	Member States
A	North	Denmark, Estonia, Finland, Latvia, Lithuania and Sweden
B	Centre	Austria, Belgium, Croatia [*] , Czech Republic, Germany, Hungary, Ireland, Luxembourg, the Netherlands, Poland, Romania, Slovenia, Slovakia and the United Kingdom
C	South	Bulgaria, Cyprus, France, Greece, Italy, Malta, Portugal and Spain

^{*} as a new EU MS Croatia is not included in original zoning of Regulation (EC) No 1107/2009

11 Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC; OJ 24/11/2009 L 309, p.1-50.

Soybean varieties suited for the northern zone (A) mature quickly, in about 80-90 days after planting, whereas those for the southern warm climate mature slower in 100-150 days. Maturity groups are designated from 000 (very early), thorough 00, 0, I, II, III and IV (very late). Depending on the type of cultivar (very early to late) the temperature sum (sum of the temperatures of days with a mean temperature above 10°C) for the soybean vegetation period is in the range of 1,200-1,750° days. Not only the region, but also the sowing date determines the choice of the optimal maturity group. Early varieties are less sensitive to photoperiod. In Austria (central zone, B) well adapted early maturing cultivars of group 00 and 000 are used (Bäck, 2010), meaning they need less days to reach maturity than later groups because day length during growth is long (May until September). For the southern zone (C), soybean cultivars are chosen from maturity groups 000 to II and 0 to II in France and Italy, respectively. Since France also has regions

representing climatic conditions typical for the central zone, the cultivation of 000 type varieties is recommended in the northern and eastern areas of France (CETIOM, 2014).

Pod development of soybean starts in late summer (August) and harvesting is normally carried out from September to October. During pod dehiscence (Anderson and Vicente, 2010) soybean seeds may be mechanically dispersed, facilitated by water and occasionally by birds.

Table 2 summarizes the number of soybean varieties listed in national catalogues in some of the EU MS. In total, presently 367 soybean varieties are registered in the EU Common Catalogue (<http://ec.europa.eu/food/plant/propagation/catalogues/database/public/index.cfm?event=RunSearch>) and their seed can be marketed throughout the EFTA (European Free Trade Association) countries.

Table 2: Soybean varieties listed in national catalogues of EU MS for 2013*

Member State	Number of registered varieties	Maturity classes	Growth habit
Austria	1 19 26 1	0000 000 00 0	75% indeterminate; no determinate varieties
Bulgaria	6	early and precociousness varieties	no data
Croatia	31	00 - II	indeterminate
Czech Republic	3 5 3	000 00 I	semi-determinate, semi-determinate to indeterminate
France**	10 15 3 17 2	000 00 0 I II	no data
Germany	3	00 - 000	no data
Hungary	58	00-II	mostly indeterminate
Lithuania	1	early maturity	indeterminate
Netherlands	2	000	semi-determinate
Slovakia	1 2 5 3 4 2	00 0 I II III IV	no data
Spain	1 5 2	I II III	no data
United Kingdom	2 5 1	0000 000 00	no data

* data are provided by members of TWG-Soybean 2 - only list A

** Only list A

3. Soybean Cultivation in the EU: demand and crop production

Soybean production for 2013 in the EU-28 is expected to be about 1.228 million tons, which is 31% more than in 2012 (939.1 thousand tons) (COCERAL oilseed crop forecasts, June 2013¹²). That corresponds to an increase from 3.45% to 4.25% of total oilseeds production in EU.

The worldwide production of soybean has increased in the past 40 years by more than 500%. World production of soybeans in 2012/2013 was 267.58 million tons and according to forecasts a further increase is expected. Soybean represents 57% of the total world production of major oilseeds (472.08 million tons). The United States of America (USA) accounts for 35% of the world's soybean production followed by Brazil, Argentina, China and India (FAOSTAT, 2010). Additionally, soybean is the worldwide leading GM crop. In 2012 78 % (Clive, 2013) of the cultivated soybeans were GM. In this same year, 85% of the soybean production in the USA, 98% in Argentina and 64% in Brazil was GM (EU agriculture – Statistical and economic information 2013).

The main factors driving the growing world production of soybean are on the one hand the higher standard of living in countries like China, which results in an increasing demand for meat and thus for animal feed, and on the other hand a higher demand for biodiesel feedstocks (Soyatech, 2012).

However, in 2011 the EU soybean production comprises only 2% of the EU-27 consumption of soybean meal, which was about 31 million tonnes per year (COCERAL, 2011). 68% of the EU-27 soybean meal consumption is covered directly from import and 30% is produced from imported soybean. According to the COCERAL trade forecasts 2013/2014 (August, 2013) import of soy meal into the EU will be 20.1 million tons corresponding to an increase of 2 million tons in comparison to the year 2012/2013. Import of soybean oil to the EU is according to forecasts the same as in 2012/2013 (300,000 tons). Import of soybeans will reach 12.1 million tons (74% of imported major oilseeds to the EU), which is a slight decrease in comparison to the 12.25 million tons in 2012/2013.

Almost 80% of the vegetable proteins (COPA-COGECA, 2011) used for feed in the EU is imported, and within these imports, 75% of the material is GM, mainly from South America. This amount of imports represents a cultivated area of about 17 million ha, equivalent to the EU-15 wheat area.

The largest soybean producers in the EU are Italy, Romania, Croatia, France, Austria and Hungary accounting for more than 80% of the EU soybean cultivation area. The soybean cultivation area in these MS is shown in table 3.

Table 3: The top 6 EU-MS for 2010 cultivated soybean (Eurostat, 2011)

Country	Cultivated area
Italy	159 000 ha
Romania	65 200 ha
Croatia*	58 896 ha
France	50 900 ha
Austria	34 400 ha
Hungary	33 500 ha

* FAOstat, 2011

12 (COCERAL) – Committee of the Cereals, Animal Feed, Oilseeds, Olive Oil, Oils and Fats and Agrosupply Trade of the EU, 2013-06-20

In Italy, soybeans are produced in the northern regions of the country, with the region of Veneto accounting for 50% of Italy's total soybean production. In France soybean fields are concentrated in Midi-Pyrénées (Bassin méditerranéen), Aquitaine, Languedoc-Roussillon, Rhône-Alpes, Franche-Comté, Alsace and Poitou-Charentes. In Romania, before accession to the EU in 2007, GM soybeans were cultivated on 137,300 hectares of a total 190,800 hectares of soybean cultivation in 2006. Due to the ban on growing GM soybean after accession, acreage declined to 49,000 ha and increased again in 2010 to 63,424 ha, representing a total soybean production of 149,940 t (FAOstat).

In Austria soybean production¹³ in 2013 covered 42,027 ha and was concentrated mostly in the Federal Provinces Burgenland (13,683 ha), Upper Austria (12,552 ha), and Lower Austria (8,626 ha).

In Hungary, about 60% of the soybean fields can be found in the southwest of the country, the biggest sown area is in Baranya County. Approximately 21-25% of the soybean cultivation takes place in the south-eastern Bács-Kiskun County.

In the Czech Republic soybean is grown in warm areas, which are typical for growing maize or beets. Most of the areas where soybean is cultivated are located in central Bohemia and central Moravia.

In Bulgaria the main regions of soybean cultivation cover the northern part of the Danube plain, areas of the middle part of the Thracian lowlands and the southern coast with an altitude of up to 150-200 m as well as the middle part of the Danube plain, Dobrudzha, Thracian lowlands and Northern Black Sea coast with an altitude of up to 300- 350 m.

There is an increasing interest in soybean cultivation in Germany. For years cultivation area was around 1,000 ha, but it has risen continuously since 2009. In 2013, soybean area increased to 6,500 ha due to the demand of GM free soybeans. Around 4,000 ha were grown in Bavaria, about 2,000 ha in Baden-Württemberg. A relatively fast expansion of cultivation area is taking place in Unterfranken and in the Rhine valley, whereas the expansion to the North is slower.

Soybean cultivation in Spain is testimonial; only 534 hectares were grown in 2013. The region of Extremadura accounts for 90% of this cultivated area.

In Lithuania the cultivation of soybean is distributed all over the country. About 30% of soybean crops are cultivated in the northern part of the country.

Of the limited amount of soybean produced in the UK, the majority is grown in the south of England. The small amounts produced in other areas, such as the east of England, are generally grown under plastic.

In the Netherlands, feasibility of commercial soybean cultivation is still being tested by farmers in an Agrifirm project from 2011 onwards, with 26 ha in 2013 and 100 ha in 2014.

The main factor limiting expansion of the soybean cultivation area in Europe is a too low temperature sum during cultivation due to the lack of solar irradiation and too low temperatures (below 10°C) during autumn in the northern part of the continent.

Soybean favours warmer climates and therefore also may profit from climate change as far as water availability does not become a limiting factor. Lane and Jarvis (2007) evaluated the impact of climate change on a variety of crops, including soybean, by applying the Ecocrop model (<http://ecocrop.fao.org/>). This modelling predicts a gain of global area suitable for soybean cultivation in 2055 that reaches 14% and covers the north and centre zones (A and B) of Europe. As well the currently most suitable zone of Europe, the south will remain suitable with adoption of soybean cultivars with higher maturity classes compared to the currently used ones.

A recent initiative to increase the soybean cultivation area in the EU is the Danube Soya Declaration, an initiative of the Austrian-based Association „Danube Soya”, which promotes cultivation of GM-free soybean to meet the domestic demand of the livestock industry and to develop the Danube region as a protein supply core of Europe. The Declaration is signed by: Austria, Croatia, Bosnia & Herzegovina, Serbia, Hungary, Slovenia, Switzerland, Bavaria and Bulgaria. (<http://www.donausoja.org/donau-soja>)

Concerning size of the farms cultivating soybean big differences between MS exist (Eurostat). In Austria, Bulgaria, Germany, Italy and Netherlands rather modest acreages per farm are noticed (2-20 ha). In France, Romania and Hungary big farms are dominant (>100 ha).

¹³ Statistik Austria 2014: http://www.statistik.at/web_de/statistiken/land_und_forstwirtschaft/agrarstruktur_flaechen_ertraege/bodennutzung/index.html

4. Existing segregation systems in soybean production

4.1. Soybean seed production

Soybean seed production in the EU mainly takes place in Italy, Croatia, Hungary, Austria, France and Romania. In 2011 the amounts of certified soybean seed produced in these countries were 12,995 t in Italy, 5,420 t in Croatia, 3,770 t in Hungary, 3,690 t in Austria, 3,348 t in France, and 2,651 t in Romania (data available only for 2009), respectively¹⁴. The significant part of the breeding material for the production of certified seeds (i.e. pre-basic seeds) mainly originates from the US (Ceddia and Rodríguez-Cerezo, 2008). Recently the Danube Soy Initiative launched studies¹⁵ for the evaluation of soybean breeding activities in Europe and the screening and collection of local genetic resources (phenotypic and genomic) of soybean in order to broaden existing genetic diversity and to stimulate regional breeding activities.

The soybean seed production in Italy has been introduced from North America during the 1980s. Over the years it established itself in the northern regions of Italy.

The volume of soybean seed production in Italy partially reflects the dynamics of the certified area. However, the volume of seeds certified in any year also includes seeds stored from previous years and/or seeds produced abroad. Other factors that affect the volume of certified seeds are growing conditions. So for example, the decline in the volume of seeds certified in 2003 despite of the increase in the certified area reflects the bad growing conditions due to the drought.

With respect to the area, in 2005 pre-basic and basic seeds in Italy accounted for over 50% of the certified area with 2,635 ha. In terms of volume though, basic and pre-basic seed production in Italy only accounted for 13% of the total amount of the produced certified seeds with 1,361 tons in 2005. In Italy the increase in the area and volume of certified seed production has also been accompanied by a decline

in the use of farm-saved seeds. Before 2000 farm-saved seeds accounted for up to 20-30% of the seeds used by farmers, while today they represent less than 5%.

For Romania after the discontinuing of HT (herbicide tolerant) GM soybean cultivation in 2007 data are not available from ESCAA.

Production of certified soybean seed in France was carried out on an area of 1,700 ha in 2010 to which an area of approximately 10% has to be added for previous generations, basic and pre-basic seed (P. Rogani 2013, com. pers.) and accounts in total 3,719 t. Production areas are mainly located in the Rhône-Alpes, Aquitaine, Midi-Pyrénées, Franche-Comté, and Burgundy, with engagement of about 200 multipliers farms.

The production of soybean seeds¹⁶ takes place in Austria in Lower Austria (1,534.18 ha certified and 580.55 ha basic/pre-basic), Upper Austria (575.99 ha/264.73 ha), Carinthia (505.59 ha/0), Burgenland (142.86 ha/21.13 ha), and Salzburg (133.67 ha/10.72 ha; Data: 2013; Source: BAES 2013).

In Hungary the area for soybean seed production has ranged between 2,000-3,300 hectares since 2004. The sector produced between 2,400-5,300 tons of certified seeds per year in this period.

In Germany, soybean seeds are mainly imported from Austria and France. Soybean seed production is limited to an area of about 100 ha and is concentrated in the southern Federal States Bavaria and Baden-Württemberg.

Usually the first stage of soybean seed production takes place in seed companies, where pre-basic seeds (G1, G2 and G3 generation) are multiplied from parental lines (G0). After that, from the pre-basic seeds (G2 or G3) which are transferred to multiplier farmers, basic seeds (G4) are produced and then certified seed (R1).

¹⁴ Data of European Seed Certification Agencies Association (ESCAA)

¹⁵ Danube Soya Initiative, Congress report 2012: http://www.donausoja.org/uploads/media_files/document/orig/066/066_60_e1b95cfd20894c78cc7751fd5db2629dfaa03222.pdf

¹⁶ BAES 2013: <http://www.baes.gv.at/saat-pflanzgut/statistiken/feldanerkennungsflaechen/>

For seed production, soybean as a self-pollinating plant species requires smaller minimum population sizes (minimum number of plants needed to ensure genetic integrity) and shorter isolation distances (distance needed between same varieties to avoid crossing) compared to cross-pollinating species.

The isolation distances for soybean seed production fixed to meet the varietal purity standards, established by Council Directive 2002/57/EC on the marketing of seed of oil and fibre plants¹⁷, reported by Le Ny et al. (2011) for soybean cultivation are as follows:

- Pre-basic and basic seeds (G0 to G4): 5 meters to the same variety and 10 meters to a different variety. The rate of tolerated varietal impurities is 0.5%.
- Certified seeds marketed (R1): 1 meter to the same variety and 5 meters to a different variety. The rate of tolerated varietal impurities is 1%.

In order to prevent mechanical mixing (Mallory-Smith and Zapiola, 2008) the minimum distance between soybean multiplication fields and neighbouring crops with similar seed size is required to be at least 40 cm in Austria¹⁸.

In the case of soybean seed production in Brazil, to meet a 1% threshold requirement for labeling (Brasil, 2003), Schuster (2013) reviewed all possible sources of admixture and concluded that an isolation distance of 3 m between different cultivars is sufficient. For non-GM seed production with a tolerated threshold of 0.1%, he recommended an isolation distance of at least 5 m, and to increase the distance to 8 m in regions with insects of the order *Hymenoptera*.

4.2. Case study of Coexistence of GMO and non-GMO soybean in France

In 2011 the Scientific Committee of the High Council of Biotechnology of France published an expert report on the coexistence of GMO and non-GMO, including a case study for soybean cultivation (Le Ny et al., 2011). It covers all stages of soybean production: sowing, fertilization, crop protection (weeds, fungal diseases and pests), harvesting, and storage. It considers all factors that may lead to impurities, such as pollen mediated gene flow, seed dispersal, volunteers, and accidental mixtures.

The main conclusion of the report is that the actual practice of soybean seed and crop production will not lead to GMO adventitious admixture exceeding 0.9% (in the case of a single gene event) but respecting the 0.1% threshold implies the implementation of a set of coexistence measures.

Spatial isolation remains a feasible coexistence measure to ensure very low cross-pollination. However, given the small contribution of outcrossing to soybean impurities, spatial isolation would only marginally improve the rate of adventitious presence of GMO in conventional crops. Since plants at the field edges are more sensitive to pollen-mediated gene flow, separate harvest of field edges is an effective measure for limiting adventitious GM presence, but usually is not needed in soybean if field distances of 10 meters between plots exist.

A temporal isolation in a given region is difficult to achieve, because varietal choice is limited to one maturity group or close maturity groups. Therefore coincidence of flowering cannot reliably be avoided (P. Jeanson, pers. com.).

The cleanliness of equipment and facilities through which soybean seeds pass is the essential condition for reducing mixtures occurring on the farm and entering the supply chain. For seeds, efforts need to focus on the operations taking place in the processing plant.

The threshold of 0.1% adventitious GM presence cannot be achieved by the existing scheme for seed production, according to the adopted variety purity requirements (impurity in certified seeds, less than 1%, Council Directive 2002/57/EC). If these botanical impurities comprise of outcrosses resulting from cross-pollination with a single gene GM counterpart, each seed resulting from cross-pollination will contain 47.5% transgene DNA, because soybean seeds have no endosperm and consist of 5% integument and 95% embryo (Holst-Jensen et al, 2006). Therefore, the varietal purity standards of certified soybean seeds, obtained by existing seed production schemes would theoretically limit the GM admixture to a maximum of around 0.5% (0.24% and 0.48% for basic and certified seeds, respectively). Achieving a threshold of 0.1% would require the implementation of coexistence measures at critical points on farm (cleanliness of machinery, storage, isolation distances) and during seed lot preparation (to avoid admixture during production in the processing plant).

To avoid exceeding 0.1% GM admixture in non-GM soybean lots, Le Ny et al. (2011) recommended a set of coexistence measures:

- spatial isolation: 10 meters from any GM field for seed productions;
- Thorough cleaning of all machinery (seed drill, combine, trailers, etc.);
- Dedicated silo and dryer if storage of soybean in the farm;

¹⁷ Council Directive 2002/57/EC of 13 June 2002 on the marketing of seed of oil and fibre plants. OJ L 193, 20.7.2002, p. 74

¹⁸ Sorten und Saatgutblatt Sondernummer 36, Republik Österreich - http://www.baes.gv.at/fileadmin/_migrated/content_uploads/Feldanerkennung_Gro%C3%9Fsamige_Leguminosen_01.pdf

- Proper verifications of seed bags before seed production (variety, generation);
- Spatial or temporal isolation in the seed processing plant during sorting /processing phase;
- Molecular analyses (PCR) starting from seed production throughout the whole production chain.

4.3. Canadian Identity Preserved Recognition System (CIPRS)

Identity Preservation (IP) means maintaining a crop's unique traits or quality characteristics from seed through crop production, storage, transportation, handling and processing.

The Canadian Grain Commission introduced in 2004 the Canadian Identity Preserved Recognition System (CIPRS) as a voluntary program, which certifies the effectiveness of a company's identity preserved system of specialty grains, oilseeds or pulses. CIPRS is a voluntary system of process verification and certification. It is coordinated by the grain companies or traders and is market driven, not regulatory. The added value of IP crop is granted by price premiums. This price incentive keeps IP systems self-regulating.

In Canada, nearly 200 soybean varieties are grown, each with its own tolerance for specific climatic conditions and soil traits and resistance to certain crop diseases and pests. Each variety also produces soybeans with varying characteristics, e.g. higher protein, sugar or oil content as well as differences in flavour. Taste and consistency traits are critical to companies seeking to create a consistent product, and they are willing to pay a premium price for that assurance.

The IP procedures for soybean production were developed by the Canadian Soybean Exporters Association¹⁹ and have two purposes. On one hand it shall ensure IP for the export of non-GM soybean, on the other hand the CIPRS shall guarantee production of premium quality soybean.

The system relies on third party verifications to gain credibility. The verification is provided by the Canadian Grain Commission or other certified accreditation bodies.

The CIPRS provides third party verification of the processes the Canadian industry uses to deliver the specific quality attributes that domestic and international buyers are demanding.

The certified companies that sell products through IP programs have quality assurance and traceability systems

for the production, handling and transportation of specialty grains, oilseeds or pulses throughout the entire value chain.

Canada's Identity Preservation Standard is a completely integrated system of identity preservation with an unmatched record of delivering specific soybeans to specific purchasers, ensuring complete traceability from purchasing seed to the sealing of shipping containers. The Standard's strict rules provide customers with the assurance that the soybeans they receive have exactly the ordered quality.

CIPRS also ensures that a company's quality management system meets the standard created by the Canadian Grain Commission, a standard that is compatible with the globally recognized International Organization for Standardization (ISO) system.

In CIPRS the maintaining of IP requirements established by soybean industry on farm level is achieved by utilization of:

- Certified Seed;
- Approved isolation distances;
- Field history;
- Cleaned planting & harvesting equipment;
- Cleaned & labeled storage bins;
- Cleaned trucks/trailers.

The seeds used for the production of IP soybean must be certified or of equivalent quality. Equivalent seed must be produced under a controlled system similar to the Canadian Seed Growers' Association pedigreed seed increase system²⁰. Grower must retain his/her invoice or receipt for each lot of seed purchased to produce the quantity of Identity Preserved (IP) soybeans being contracted or delivered.

The approved minimum isolation distance is 3 meters from other soybean and pulse crops, which is sufficient for achieving the market requirements for tolerance of GM presence in non-GM soybean from 0.5 to 1.0%. The utilization of a proper isolation distance must be verified and documented at the time of field inspection. There is no isolation distance necessary between IP soybeans and cereals, canary seed or flaxseed providing the crops do not overlap.

Pedigree soybeans must not be grown on a field which in the previous year was used for the production of non-pedigree soybeans or a different soybean variety. The grower must provide records for field history use.

¹⁹ <http://www.canadiansoybeans.com/content.php?id=12>

²⁰ http://seedgrowers.ca/wp-content/uploads/Circ6_Complete_English.pdf

All equipment used in the IP soybean production of pedigree seed, including planting, harvesting, transportation and on farm storage must be cleaned thoroughly before use, particularly if it has been used previously for a different variety or kind of seed or grain. Growers must sign a document to authenticate that the equipment was cleaned prior to using for production of pedigreed soybean seed.

5. Review of the available information on adventitious GM presence in soybean crop production

5.1. Seed impurities

The purity of soybean seeds is of significant importance for the purity of soybean harvests. It is evident that the purity of the seed stock must equal or exceed the purity standards of the final product. Therefore the presence of GM seeds in conventional seed lots is a critical factor and must be managed to achieve coexistence. It is obvious that the best approach to manage this is the use of certified soybean seeds that comply with legal EU regulations.

The two important parts of EU legislation covering the purity requirements of soybean seeds are the Council Directive 2002/57/EC on the marketing of seed of oil and fibre plants and Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

In annex II of the Council Directive 2002/57/EC the conditions which must be satisfied by marketed soybean seeds are set up. Basic seeds must have a varietal purity not less than 99.5% and certified seeds not less than 99.0%.

In terms of adventitious GM presence, there are no tolerance thresholds (for authorised or unauthorised GM events) for conventional soybean seeds marketed in the EU, therefore marketed conventional soybean seed complying with EU legislation will not be a significant source of adventitious GM presence in the final crop. Table 4 overviews data from some MS about the quantities of produced certified soybean seed in a relation to the overall production of certified seeds per MS and the number of control samples for examination of GMO presence taken from all certified seed lots and in particular from certified soybean.

Table 4: Control inspections for GMO admixture in certified seed lots carried out by EU MS in 2013

MS	Certified seeds, t		Control samples			
	total	soybeans	total	positive of total	soybean	positive of soybeans
Austria	111 256	5 270	171	2	22	0
Bulgaria	20	5	20	0	0	0
Croatia	-	-	-	0	10	0
Czech Republic	251 309.34*	535.59*	72	3	5	2
Spain	305 432	4.18	573	51	4	0
France	1 505 950	4000	88	4	4	0
Germany	-	-	968	13(maize)	19	0
Lithuania	41 857	0	6	0	0	0

*in 2012

Note: In the UK the GM Inspectorate carries out voluntary audits of companies marketing conventional soybean seed. These annual audits examine the controls companies have in place to minimise the risk of adventitious GM presence in their seed. Control samples are only taken and tested if there is a suspicion of GM presence. In 2013, all audited companies were judged to have satisfactory controls in place. There was no suspicion of GM presence in any soybean seed lots, hence no seed was sampled and tested.

5.2. Cultivation

5.2.1. Outcrossing to wild relatives

In general, wild annual species of the subgenus *Soja* as well as wild perennial species of the subgenus *Glycine* are candidates for gene exchange with cultivated soybeans. No other species is closely enough related to cultivated soybeans to enable outcrossing. However the wild soybean species are endemic in China, Korea, Japan, Taiwan and the Far East of the Russian Federation, but do not naturally exist in Europe (Zukovskij, 1950 and Hymowitz, 1970). Therefore, the spread of transgenes by crossing with wild plants followed by backcrossing to cultivated soybean varieties is not a matter of concern in EU countries.

5.2.2. Outcrossing between GM and non-GM soybeans

Yoshimura (2011) investigated pollen-mediated gene flow from GM to non-GM soybean cultivars caused by wind. The airborne soybean pollen was sampled using Durham pollen samplers located in the range of 20 m from the field edge. The dispersal distance was assessed in a wind tunnel under constant airflow and it was compared with the anticipated distances based on the pollen diameter. Little airborne pollen in and around the field was detected and the dispersal was restricted to a small area at the field edge even when soybean flowers were in full bloom. Considering soybean characteristics with a stigma invisible from the outside and a short pollen life, wind-mediated pollination in soybeans appears to be negligible.

The natural cross-pollination rate in cultivated soybean has been extensively investigated in several studies carried out under different environmental conditions in USA, Japan, and Brazil (Ray et al., 2003; Yoshimura et al., 2006; Schuster et al., 2007; Pereira et al., 2007; Abud et al., 2007, and Silva and Maciel, 2010). In all cases coincidence of the flowering period of the examined soybean cultivars was observed, as well as presence of potential insect pollen vectors for gene flow as honey bees and others pollinators. In one of the studies the cross-pollination rate was examined using conventional cultivars differing in flower colour (purple and white, Ray et al., 2003), the others used one conventional and one HT GM soybean variety. In the first case, the cross-pollination rate was estimated in a two year period by comparing the seed amounts of hybridized plants. The percentage of outcrossing between conventional and HT GM soybean varieties (Yoshimura et al., 2006; Schuster et al., 2007; Abud et al., 2007, Pereira et al., 2007 and Silva and Maciel, 2010) was calculated as percentage of surviving seedlings of offspring seeds after spraying them with glyphosate. The surviving seedlings were counted as heterozygous offspring.

All these studies show that outcrossing in soybean decreases very rapidly with distance from the pollen source (Table 5), with less than 0.1% in distances exceeding 2 meters. Ray et al. (2003) evaluated cross-pollination in soybean at different distances, and found outcrossing rates from 0.41% to 0.03% for 0.90 m to 5.4 m from the pollen source. Gene flow between plants in the same row, interleaved and spaced 15 cm, was 1.8%.

To gather information about the possible influences of the climatic conditions, Yoshimura et al. (2006) performed a four year experiment. The highest outcrossing rate observed between transgenic and conventional soybean varieties was 0.19% at a distance of 0.7 m. Over the four years, the furthest distance that pollen travelled was 7 m. The authors concluded that the pollen densities, as well as the anatomical features and short life span of soybean pollen, result in the possibility of out-crossing by wind being minimal, and that the main cause of cross-pollination in soybean is the presence of insects, particularly Hymenoptera.

Abud et al. (2003) found rates of gene flow from 0.44% to 0.45% for 0.5 m separation, and 0.04 to 0.14% for 1 m separation. In another study, Abud et al. (2007) reported outcrossing rates of 0.52% between GM HT and non-GM soybeans at a distance of 1 m and 0.12% at a distance of 2 m. The authors considered that a distance of 10 m between GM and non-GM soybeans is sufficient to prevent adventitious presence by pollen flow.

Schuster et al. (2007), obtained an outcrossing rate between transgenic and non-transgenic soybeans of 0.61% for 1 m, and this rate was reduced significantly to 0.29% at 2 m. These authors concluded that with a distance of 8 m no gene flow between soybeans plants via pollen occurs.

Pereira et al. (2007) evaluated the outcrossing rate between transgenic and non-transgenic soybean varieties at two locations in Brazil. The outcrossing rate in Forest-MG was 1.27% and in Viçosa-MG 0.25% for the rows at a distance of 0.5 m from the pollen source. In Viçosa-MG, outcrossing was observed only up to 2 m distance from the pollen source, and in Forest-MG up to 4 m. In another study, Pereira et al. (2012) concluded that gene flow among soybean varieties is rare at a distance farther than 3 m.

Silva and Maciel (2010) also evaluated the pollen flow between transgenic and conventional soybean plants and obtained outcrossing rates of 0.025% for 1 m distance and 0.01% for 2 m.

Virtually all of these studies (table 5) agree on the absence of any detectable outcrossing at a distance of 10 meters.

Table 5: Cross-pollination rate between soybean varieties

Growing location	Test system	Distance	Cross-pollination rate	Reference
USA (Mississippi Delta)	flower color	0.15 m 0.9 m 5.4 m	1.8% 0.41% 0.03%	Ray et al. , 2003
Japan	GM and non-GM	0.7 m 7 m (max) 10.5 m	0.19% (max) 0.04% 0%	Yoshimura et al., 2006
Brazil (Planaltina, DF)	GM and non-GM	0.5 m 1 m	0.45% 0.14%	Abud et al., 2003
Brazil (Planaltina, DF)	GM and non-GM	1 m 2 m 10 m	0.52% 0.12% 0%	Abud et al., 2007
Brazil (Cascavel, PR)	GM and non-GM	1 m 2 m 3 m 4 m 5 m >8 m	0.61% 0.29% 0.23% 0.22% 0.23% 0%	Schuster et al., 2007
Brazil (Florestal, MG,) (Viçosa, MG)	GM and non-GM	1 m > 4 m 1 m > 2 m	1.27% 0% 0.25% 0%	Pereira et al., 2007
Brazil (Alfenas, MG)	GM and non-GM	1 m >2 m	0.25% 0.01%	Silva and Maciel, 2010

5.2.3. Volunteers

Although dispersed seeds may germinate and grow as a volunteer in the year following cultivation, the soybean seeds have no innate dormancy (which is selected for in commercial soybean seed) and germinate quickly as soon as the soil temperature exceeds 10°C and soil moisture is adequate (Anderson and Vicente, 2010). However under European conditions temperatures during autumn and especially during winter drop significantly below 10°C, which make the survival of soybean volunteers almost impossible due to their cold sensitiveness (ref. to section 1. Soybean biology). Even if soybean volunteers do appear they demonstrate no invasive behaviour and little competitiveness to other cultivated or wild plants. Suitable crop rotation systems like soybean-maize or soybean-maize-wheat also contribute to the elimination of soybean volunteers in addition to their well-documented benefits in respect to crop productivity (Meese et al., 1991; Lund et al., 1993), numbers of nematodes (Howard et al., 1998), and disease and pest control (Pikul et al., 2005). Additionally, soybean volunteers can easily be controlled mechanically or chemically (Bond and Walker, 2009).

5.3. Process management during sowing, harvesting, drying and storage in farm

On farm processes which can lead to impurities of grain lots include leftover grain in planters, combines, grain augers, trucks, silos and dryers. Seed dispersal may also occur during seeding, harvesting, handling, storage and transport.

The management of these potential sources of adventitious GM presence differs in their complexity and consequently in the possibility for their control. For example the internal mechanical complexity of a combine makes it more difficult to control or predict GM admixture compared to those from other sources such as grain handling, storage equipment, or the planter. Therefore the adoption of an adequate machinery cleanout is advisable, to achieve levels of soybean grain purity required for marketing (Schuster, 2013).

5.3.1. Sowing

To avoid possible mixing during sowing, seed planters should be cleaned from previously sowed seed lots as reported by Messean et al. (2006). Cleaning recommendations depend on the type of seed planter and seed metering mechanism (Hanna et al., 2004). For specific procedures for individual planters, operators have to refer to the operation manuals. Small numbers of seeds may stick somewhere inside the seed planter and later drop out over a short distance in a row at a random time (Hanna et al., 2002). However, it is hard to predict how the remaining seeds will exit the seed planter, individually over a long distance or as a concentrated lot at

an unknown time and location. Experience with an individual planter over time will help to find where seed may be lodged.

5.3.2. Harvesting and seed dispersal

Harvesting is the most critical step, since combine harvesters are in general a primary source of on-farm grain comingling. Due to its complex construction, a complete cleaning of the machine is nearly impossible and uneconomic. For example Hanna et al (2006) reported that the total material remaining in the combine ranged from 38 to 84 kg, 61% of which was whole grain. The greatest amounts of soybean material (8 to 34 kg) were found in the grain tank and rock trap. Intermediate amounts of soybean were found in the head or feederhouse, elevators, the cylinder/rotor, the unloading auger, and rear axle/chopper area. The least amounts were found in the cleaning shoe and straw walkers (cylinder-type machine).

However, it is difficult to estimate exactly how much of this remaining material will end up in the next crop. Preliminary research shows that comingling occurs to some extent even after the “initial flush” of a new crop has moved through. While comingling will still happen in subsequent loads, its rate decreases very rapidly. The first hopper load (approximately 2.5 - 5.5 tonnes) removed the majority of the previous crop, with trace amounts in hopper two and none detected in hopper three (Hanna et al., 2004 and Ess et al., 2005). However, a thorough cleaning of the combine harvester from front-to-back and top-to-bottom is recommended if the harvester is used for non-GM soybeans after harvesting of GM soybeans (Hanna et al., 2002). Cleaning a combine can take anywhere from a few minutes to many hours, depending on the technique used and the extent of cleaning that is aimed at. When cleaning a combine, it is important to consider where the greatest chances are for previous grain commingles with the new grain. For example, it is very likely that any material in the header and feederhouse will quickly dislodge and travel through the combine during the next crop. Fortunately, these areas are fairly easily cleaned with compressed air.

Choosing the appropriate technique for combine clean-up should be based on the desired level of purity of the grain.

5.3.3. Transport, storage and drying

Although soybean seeds are quite big and this characteristic should ease the handling during these steps, the cleaning of trucks and trailers is required when non-GM grains are handled after GM lots. Presently no published data for soybean concerning the possible levels of admixture due to transport and on-farm storage were found.

Silos and other storage space must be thoroughly cleaned and inspected after emptying of GM crops and prior to storing of non-GM ones.

In general, GM and non-GM grain lots should be transported and stored separately.

Soybean drying is crucial when the grains were harvested with moisture contents above 16%, and is usually performed as soon as possible to avoid grain deterioration and microbial infections.

There are no additional best practice recommendations in respect to minimize potential GMO admixture. However, it should be assured that lots of GM and non-GM soybean are dried separately. After drying of GM lots the grain dryer has to be thoroughly cleaned.

6. Occurrence of soybean material in honey

6.1. Soybean attractiveness for honeybees

Cultivated soybean are not very attractive for bee species (Blickenstaff and Huggans, 1962). However, the visit of soybean flowers by bees for both pollen and nectar harvesting is reported. Cultivated soybean is visited by short-tongued bees from the families *Apidae*, *Megachilidae*, *Halictidae*, *Anthophoridae*, and *Adrenidae* (Erickson, 1975; Chang and Kiang, 1987; Ortiz-Perez et al., 2007; Perez et al., 2009). Pollinators from the order *Lepidoptera* have also been observed on soybean (Chiari et al., 2005).

The attraction for pollinating insects is determined by flower colour, flower accommodation, flower anthesis and pollen dehiscence, volatile production, as well as nectary structures and secretion.

The nectar of soybean is a complex mixture of many compounds, consisting of sugars, amino acids, proteins, lipids, and other compounds that provide nutritional and protective functions (Perret et al., 2001; Carter et al., 2006 and Horner et al., 2003). Honeybees visit soybean flowers mainly for nectar collection, which has a sugar content between 37 and 45% (Erickson, 1975; Chiari et al., 2005). Nectar secretion of soybean flowers increased as day air temperatures at which plants were grown increased from 20 to 32°C (Robacker et al., 1983). It was shown also that plants grown at higher temperatures are more attractive to bees than those grown at lower temperatures of maximal 29°C (Robacker et al., 1983). These differences in nectar quantity could be a reason for the described variation in attractiveness of different soybean varieties for honey bees (Erickson et al., 1978). For example *G. falcate* and *G. canescens* species are different in nectar composition from the other *Glycine species* (Brown et al., 2002; Doyle et al., 2002, 2003, 2004).

Even though the quantity of nectar produced by a single soybean flower is small, due to the high flower density (up to 800 flowers in the plant's lifespan) and the big cultivation area, soybeans could be a good food source for bees. However each soybean flower lasts only 1 day and zygomorphic

flowers are hermaphrodite and self-fertile (for details see section 2.1. Flower and pollen morphology). This questions the efficiency and extent of soybean pollen transfer in honey.

6.2. Soybean material in honey

Lieux (1972 and 1981) performed an extensive, large scale melissopalynological study of commercial honey produced in Louisiana and Mississippi (USA), analysing 54 and 68 samples respectively. In both states soybean cultivation is widespread.

Soybean pollen grains were found in 29 of 68 samples from Mississippi and were classified as predominant in 10 samples and as secondary in three. The average content of soybean pollen grains in honey was 15% of the total pollen. The maximum content was found in certain unifloral honey samples located adjacent to soybean fields when honey was harvested immediately after the end of the soybean blooming period, being so-called seasonal honey or spring honey.

The pollen analyses of 54 Louisiana honeys (Lieux, 1972) confirmed that soybean can be an important nectar source for unifloral spring honey, harvested immediately after the end of soybean blooming.

Gallez et al. (2005) examined the content of soybean pollen in honey produced in the centre-west area of the Pampa in Argentina where large scale soybean cultivation takes place. By melissopalynological analysis the presence of soybean pollen in all examined 36 honey samples could be shown. In 97% of the analysed samples the soybean pollen was categorized as trace (<3% of total pollen) and in 3% of them as minor pollen (3-15% of total pollen).

Villanueva-Gutierrez et al. (2014) reported results of an analysis of 9 samples of honey produced on the Yucatan peninsula in Mexico. However each of these samples represents a different distance between apiary and soybean field, covering an area of 40 m to 48 km, without repetitions. The samples were obtained directly from beehives, immediately after the end of soybean blooming.

Examined soybean pollen content ranged from 48 - 8% of total pollen for honey samples taken from 40 m to 300 m distance between apiary and soybean fields. Two of these samples gave positive PCR signals for presence of GM soybean material. However, in a second sample taken from 300 m, soybean pollen could not be detected. Furthermore, no soybean pollen grains were found in samples originating from apiaries at a distance of 40 km and 48 km from soybean fields, respectively.

Similar results in respect to the presence of soybean pollen in honey, produced in the area of the Yucatan peninsula in Mexico were presented by Vides-Borrell and Vandame (2013). They reported a decrease of the soybean pollen content in total pollen from 45% to 0% for a distance of 250 to 1500 m, again with single sample measurement per each distance.

Chiari et al. (2013) studied possible differences in attractiveness of the flowers of GM and non-GM soybeans (cultivars BR-245 RR being transgenic - Roundup Ready™ and BRS- 133 being conventional) for the *Africanized* honeybee in a small scale plot trial (parcels of 24 m²). The authors could not detect any differences between transgenic or conventional soybean in respect to floral biology and attraction to insects, independent of the application of the herbicide glyphosate in transgenic soybean.

The available studies from USA, Argentina, and Mexico for presence of soybean pollen in honey indicate that soybean can provide unifloral honey, when beehives are located in the vicinity of soybean fields and honey is seasonally harvested. Soybean pollen can also be found in polyfloral honey but at the level of minor pollen or traces.

Carne et al. (1984) reported for Italy about the occurrence of unifloral soybean honey. However, no quantitative data about the presence of soybean pollen in EU produced honey are available due to the relatively minor importance of cultivation of this crop in Europe.

Another possible source of soybean material presence in honey is the use of pollen substitutes in commercial bee feeding, which contain soybean. Siede and Büchler (2001) reported about the presence of crushed soybean in different honeys from Germany, detected visually (by microscopic analysis) and by soy-specific (p35S/CTP gene cassette) PCR. However no quantitative data were provided.

Furthermore Barker (1977) and Brodschneider and Crailsheim (2010) showed that about 40% of the sugars found in soybeans, which are used as pollen substitutes, are toxic to bees. As a consequence authors suggested that isolated soybean proteins should be used as honeybee feed instead of whole meal or crushed soy.

7. Detection of GM events in soybean crops and honey

A number of methods for the detection of GM soybean have been developed. These include:

1. PCR-based methods, both qualitative and quantitative, which can also be used for the more highly processed soybean-based food products (Hubner et al., 2001; Taverniers et al, 2001; Pauli et al., 2000; Hurst et al., 1999; Terry et al., 2002; El Sanhoty et al., 2002; CropBiotech Net., 2002; Meyer et al., 1996; Vollenhofer et al., 1999; Meyer et al., 1997; Tengal et al., 2001); and
2. Protein-based methods for the detection of the EPSPS gene product in transgenic raw or unprocessed soybean products (CropBiotech Net., 2002; Meyer et al., 1996; Lipp et al., 2000; van Duijn et al, 1999)

The effectiveness of currently available immunoassay methods for detecting the presence of GM seeds in seed samples of non-GM soybean, depending of the trait, can go down even to 0.1%²¹. Mazzara et al. (2013) recently demonstrated detection properties of a GM contamination of at least 0.033%.

In 2007 the European Union Reference Laboratory for GM food and feed (EU-RL GMFF) validated a quantitative PCR method for the detection of soybean event GTS-40-3-2 (Mazzara et al., 2007). The protocol describes an event-specific real-time quantitative TaqMan[®] PCR procedure for the determination of the relative content of soybean event 40-3-2 DNA to total DNA in a sample. The method is optimised for DNA extraction from soybean seeds as well as seed containing mixtures of genetically modified and conventional soybean. The method performances are: limit of detection – $\leq 0.045\%$ and limit of quantification $\leq 0.09\%$.

In addition to this method the EU-RL GMFF has validated quantitative PCR methods for identification and quantification of several other GM soybean events²².

More methods can be found in the EU Database of Reference Methods²³ maintained by the Joint Research Centre in collaboration with the European Network of GMO Laboratories (ENGL).

When the results are primarily expressed as GM-DNA copy numbers, in most cases they need to be converted into mass fraction or vice versa. In a case of physical admixture with homozygous single gene insert soybean the conversion factor between DNA copy numbers and mass fraction is 1.0 (TGD from the EURL GMFF, 2011). However in a case of cross-pollination when half of the progeny genome comes from the transformed line (single gene GM counterpart) and half from the conventional counterpart, and considering that soybean seed has no endosperm and consists of integument (5%) and embryo (95%), conversion of number of seeds to a DNA amount (Le Ny et al., 2011 and Holst-Jensen et al, 2006) should be achieved by multiplication with a conversion factor of 0.457.

At the current state of the art of the technology a practical and robust PCR protocol able to quantify GM pollen relative to total pollen in honey is not available. The reason is that in all honeys, even if classified as unifloral, the pollen fraction consists of pollen from several species (for details please check Rizov and Rodriguez-Cerezo, 2013).

21 http://envirologix.com/artman/publish/article_324.shtml

22 http://gmo-crl.jrc.ec.europa.eu/gmomethods/search?db=gmometh&q=id%3AQT-eve-gm*

23 <http://gmo-crl.jrc.ec.europa.eu/gmomethods/>

8. Best practice for coexistence measures in soybean crop production

The TWG Soybean analysed the possible sources for potential GM admixture in soybean crop production, which are summarised in the previous sections and agreed on the following best practices for the coexistence of GM and non-GM soybean cultivation as well as honey production.

The thresholds for coexistence which were considered are the legal labelling threshold (of 0.9%) and the limit of quantification (generally accepted to be about 0.1% for routine analysis), which is required by operators in some markets. These two different coexistence thresholds are in line with the Commission Recommendation of 13 July 2010 on guidelines for the development of national coexistence measures.

8.1. Best practice for ensuring seed purity

The use of certified soybean seeds that comply with EU legislation is considered best practice since according to EU legislation any seed lot containing traces of GM material needs to be labelled and therefore can be easily identified.

In the case of cultivation of both GM and non-GM varieties on the same farm, the seeds of GM varieties should be transported to the farm and stored upon arrival in their original packaging, and separately from non-GM varieties. Label information should be retained with the seeds.

8.2. Best practice for reducing pollen-mediated gene flow

8.2.1. Isolation distances

Isolation distances are feasible and effective coexistence measures to reduce adventitious presence of GM soybean in conventional and organically produced soybean even if they are the only measure applied (worst case scenario). All available information from literature and pre-existing

segregation systems shows that to limit adventitious GM presence caused by cross-pollination to 0.9%, 5 m between the fields is enough and to achieve thresholds of 0.1%, 10 m isolation will be sufficient.

8.2.2. Temporal isolation

The replacement of isolation distances by temporal isolation by planting soybean of different maturity classes is difficult to achieve under European conditions due to the long flowering period and the limited suitability of varieties representing different maturity classes for a particular region.

8.3. Best practice during sowing, harvesting, drying and storage in farm

Harvesting is the most critical step in soybean cultivation, since combine harvesters are in general a primary source of on-farm grain comingling.

The utilization and maintenance of equipment should be done in a sound economic manner. The equipment used for processing of GM crops should be cleaned thoroughly before it can be used for processing of non-GM crops. The definition of specific recommendations for cleanout depends on type of the equipment and its construction; therefore the consultation of the operation manual is recommended. It is important to consider where the greatest chances are for previously harvested grain to commingle with the new grain and to clean these parts predominantly. Experience with individual equipment over time will help to find the most critical common areas where seed may be lodged. Additionally, choosing the appropriate technique for equipment cleaning should be based on the desired level of purity of the grain. Alternatively, the use of dedicated equipment for different production systems (GM and non-GM) or its use for non-GM crops prior to GM crops eliminates the risk of admixture.

GM crops should be stored separately from non-GM crops. The storage space must be thoroughly cleaned and inspected after emptying of GM crops and prior to storing of non-GM ones.

8.4. Best practice for coexistence with honey production

There is no available empirical data to establish a statistical relationship between soybean pollen content in honey and distance of beehives to soybean crops.

Soybean pollen is not a major fraction of total pollen in polyfloral honey. Soybean unifloral honey could reach the market if it is harvested from beehives shortly after the end of soybean blooming. Even in this case, considering the maximum pollen content (number of grains) in commercial honey and the average weight of soybean pollen grains, the weight fraction of soybean pollen in honey will definitely be below 0.1%.

In conclusion the current practices in honey production and marketing in Europe in line with quality legislation are sufficient to ensure that adventitious presence of GM soybean pollen in honey is far below the legal labelling thresholds and even below 0.1%.

9. Economic analyses of best practice

No empirical data are available to estimate the costs of implementing these coexistence best practices by EU farmers intending to grow GM soybean. However, economic data from segregation systems operating in soybean elsewhere can be relevant for this discussion and suggest that these costs are relatively small.

The economic analysis of non-GM soybean segregation and identity preservation (IP) in USA performed by Bullock and Desquilbet (2002) shows that a small fraction of farmers' total costs of segregation and IP actually comes from the steps farmers take to clean planting and harvesting equipment. For cleaning of planter and combine Bullock and Desquilbet (2002) estimated that on-farm costs per ton for non-GM soybean segregation and IP is 1 and 0.5 working hours, respectively.

Bullock and Desquilbet (2002) as well as Anderson (2005) concluded that since soybeans do not cross-pollinate, additional costs for farmers should be low due to small isolation distances needed. Isolation distance cost could be defined as the lost profit on the area bordering a crop plot on which farmers are not able to raise a crop (Gustafson, 2002). The total value of the lost area can be divided by the amount of crop yield sold to place the value on a per unit basis. Additionally, the isolation distance is a particular measure since it does not affect all farmers equally. Fields are not randomly distributed on a common physical landscape. Farmers whose neighbouring fields lie beyond isolation distance will have no constraints in their decision-making of planting GM varieties or not and will experience no economic impact at farm level. However, farmers intending to use GM varieties but with neighbouring non-GM soybean fields falling within isolation distances will be constrained in their choice. At farm level, this will have a monetary cost equivalent to the difference in gross margin between the GM and non-GM soybean varieties. At regional level, the economic effect will depend on the physical landscape area affected (Messean, 2006).

The gross margins obtained by farmers can be defined as the difference between a farmer's income and variable costs, i.e. costs that depend on production such as costs of seeds, fertilizers, pesticides, fuel used for machinery, labour etc. The above mentioned coexistence measures for spatial segregation and machinery maintenance and cleaning are accounted in partial farm budgeting as variable costs.

For example in Canada the contract premiums, which are offered to non-GM growers to compensate the costs of IP programmes (both on farm and administrative costs) and provide the economic incentive to continued coexistence of GM and non-GM production of soybeans certified for CIPRS are in a range of about C\$0.60 to C\$4.00 per ton (Anderson, 2005). In general, the costs of coexistence for GM soybean farmers would have to be compensated by monetary or non-pecuniary benefits of growing GM soybean varieties. The adoption and benefits for farmers of GM soybean cultivation in the Americas have been well studied. GM soybean is mainly adopted because of the simplification of weed control management and the flexibility that it offers to farmers. In developed countries such as the USA, GM soybean adoption is considered to be yield neutral (Qaim, 2009) and mostly neutral on farm-derived income (Fernandez-Cornejo et al., 2014). However, adoption of GM soybean by farmers is correlated with increased off-farm derived income likely associated with the flexibility and time saving gained in managing this crop. Qaim and Traxler (2005) reported an increase in total factor productivity of 10% on average soybean production for Argentina as a result of GM soybean cultivation.

This economic impact can be different in areas where weed control is not properly achieved in conventional soybean and therefore GM soybean will have a positive impact on yields. In a three-year study Vollmann et al. (2010) investigated the effects of weed pressure on yield and quality of soybean cultivars grown in Austria. In two seasons, with strong weed pressure a soybean yield was reduced by 370 and 560 kg/ha, respectively, compared with mean weed-free yield of about 2,500 kg/ha. In such high weed pressure conditions Brooks (2005) reported for Romania an average yield gain of HT soybean relative to a conventional variety of 31% for the average base yield of 2000-2500 kg/ha. Taking all of the above listed performances of GM soybean and mentioned specificity of European agro-climatic conditions into account Park et al. (2011) estimated *ex ante* an overall benefit of the adoption of GM soybean in the EU of about 10 – 38 euros per hectare. At the end, farmers will consider both monetary and non-monetary benefits of GM adoption versus coexistence costs in their decision making process to select what kind of variety to adopt.

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